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EFFECT OF CAUSTIC PASTE DISBUDDING, USING LOCAL ANAESTHESIA WITH AND WITHOUT ANALGESIA, ON BEHAVIOUR AND CORTISOL OF CALVES

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ABSTRACT
We looked at the effects of local anaesthesia with or without a non-steroidal-antiinflammatory analgesic drug (flunixin-meglumine) on behaviour and plasma cortisol after caustic paste disbudding of 1-month-old calves: at 15 min, 1, 3, 6 and 24 h (Experiment 1; n = 32); at 10, 30 and 50 min (Experiment 2; n = 35); and after local anaesthesia effect subsided (90–180 min) (Experiment 3; n = 16).
In Experiment 1, cortisol was higher at 1 h in paste-disbudded calves than in all other groups. Paste-disbudded and paste-disbudded plus local anaesthesia calves showed higher cortisol at 1 h compared with baseline values. At 15 min paste-disbudded calves showed a higher incidence of pain-related behaviours than all other groups and those with anaesthetic or anaesthetic plus analgesia showed more pain-related behaviours than controls. At 1 and 3 h both paste-disbudded and paste-disbudded plus anaesthetic calves showed more pain behaviours than controls and those with analgesic. In Experiment 2, paste-disbudded calves showed higher cortisol than all other groups at 30 and 50 min. No difference in cortisol was found between anaesthetic, anaesthetic plus analgesia and sham-disbudded calves. The incidence of pain-related behaviours was greater in paste-disbudded calves than in all other groups at all times. Calves disbudded with anaesthetic or with anaesthetic and analgesic showed more pain-related behaviours than sham-disbudded animals during the first 10 min post-procedure. In Experiment 3, paste-disbudded only calves had higher cortisol at 90 min and the anaesthetic-only group had higher cortisol at 180 min, when compared with control animals. Paste-disbudded calves showed more pain-related behaviours at 90, 120 and 150 min, and the anaesthetic-only disbudded calves at 180 min, when compared with sham-disbudded calves. In Experiment 1 and Experiment 3, several disbudded animals showed an ‘‘inert-lying’’ posture and this state may have reduced the display of the other more active behaviours.
The evidence indicates that caustic paste disbudding causes distress for at least 3 h and that local anaesthesia is efficient in controlling pain for the first hour but discomfort returns after the nerve blocking subsides. Overall, only local anaesthesia + NSAID provided effective reduction in pain as assessed by this method. Inert lying is a sign of distress in young calves after caustic paste disbudding.

INTRODUCTION
Effective disbudding of calves destroys or removes the tissues responsible for the generation of the horn. The justification for this mutilation has been that it reduces the

potential for traumas and lesions caused by horned animals and so increases safety to humans and other animals in the herd. The disbudding methods more commonly employed are: cauterization by heat, chemical cauterization (caustic paste) or amputation (scoop, knife, guillotine and saw-wire).

Paste disbudding is caused by the chemical burn of underlying tissue. The active ingredient used for disbudding is usually sodium hydroxide or calcium hydroxide. These strong alkalis cause liquefactive necrosis, resulting in saponification of fats and denaturation of proteins, which allows deeper penetration of the chemical. With caustic burns, tissue damage continues to increase as long as the active chemical is in contact with the tissue (Yano et al., 1993) and alkalis tend to penetrate deeper and cause worse burns than acids (Hettiaratchy and Dziewulski, 2004). Histopathological findings after alkali burns in pigs have revealed full-thickness epidermal necrosis and superficial dermal necrosis (Cowart et al., 2000). The pain caused by alkali is described by humans as "itching pain" or, "marked pain" (Ma et al., 2007) or sometimes as a chronic and severe pain (Kumbhat et al., 2004). Malenfant et al. (1996) found that 36% of chemical burn patients complain about pain whereas 71% of them experience paresthetic sensations. After a period of acute pain most humans affected by alkali burns develop what as been described as "neuropathic-like abnormal sensations" (Khedr et al., 1997). However, all these references deal with more extensive areas of injury than those caused by disbudding.

Morisse et al. (1995) and the EFSA report on welfare of calves (2006), assert that caustic paste is more painful than hot-iron disbudding, but Vickers et al. (2005) suggest that the contrary is true. Our previous studies (Stilwell and Lima, 2004) showed similar cortisol and behaviour results when comparing paste and hot iron, although differences in ages could have affected the results. In the United Kingdom the use of chemicals for disbudding is only permitted for calves under the age of 7 days (Animal Welfare Act, 2006). However, paste disbudding is still widely used on young calves (usually under 45 days old) in many farms around the world. In Portugal it is the most widely used method of disbudding, according to a practitioner survey (Stilwell, G., data not published). Farmers sometimes favour this method because it is easily performed and may give the idea of being less painful because there is less struggling during the procedure (personal communications). Also because there is less struggling, compared with hot-iron disbudding, it is thought to be safer for the operator and animal, although care has to be taken to prevent paste running onto face and eyes.

For the purpose of our studies we use the definition presented by Molony and Kent (1997) in which pain is define as "an aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery". Pain after disbudding has been evaluated by measuring plasma cortisol (Morisse et al., 1995; Grøndahl-Nielsen et al., 1999; Stafford et al., 2003; Stilwell and Lima, 2004; review by Stafford and Mellor, 2005). Frequency of head-shaking, ear-flicking and head-rubbing have also been used as pain indicators in studies with other methods of dehorning (Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999; Faulkner and Weary, 2000) and with paste-disbudding (Morisse et al., 1995; Vickers et al., 2005). Transitions from standing or lying have also been used previously with paste and hot-iron disbudding (Morisse et al., 1995; Vickers et al., 2005). Some studies (Stilwell and Lima, 2004; Stilwell et al., 2008) have shown that calves under 45 days old respond to intense pain by adopting an apathetic attitude presented as inert lying with head on flank and showing little response to stimuli such as those resulting from other calves. Inactivity is described as an abnormal behaviour by Wiepkema et al. (1983) and Broom and Fraser (2007). Morton and Griffiths (1985) refer general lethargy as a sign of pain in experimental animals. This behaviour has been shown in lambs after castration and described "as the time during which it was difficult to elicit any evidence of conscious awareness" (Molony et al., 1993; Molony and Kent, 1997) and is also seen in cattle and wild ruminants when young animals are left alone by their dams. All these data suggest that inert lying is closely related to high levels of distress. However, to our knowledge, very few studies have used this behaviour to assess pain in calves after disbudding. Doherty et al. (2007) assessed "inactivity behaviour" after hot-iron disbudding and Stilwell et al. (2008) used "inert lying" in the evaluation of pain in calves paste-disbudded after pre-emptive analgesia.

Previous works showed that caustic paste disbudding of young calves causes discomfort and pain for at least 4 h and suggested that local anaesthesia does not reduce pain significantly (Vickers et al., 2005). This led to the suggestion that nerve block before caustic paste disbudding is unnecessary (Duffield, 2007). The association of a NSAID with local anaesthesia has proven effective in controlling pain and distress in animals submitted to hot-iron and amputation dehorning (McMeekan et al., 1998; Faulkner and Weary, 2000; Sutherland et al., 2002; Stafford et al., 2003; Stilwell and Lima, 2004; Milligan et al., 2004). Of the few published studies on pain caused by caustic paste disbudding (Morisse et al., 1995; Vickers et al., 2005; Stilwell et al., 2008), none has concerned pain signs of calves disbudded after the injection of local anaesthesia associated with analgesia (non-steroidal-anti-inflammatory drug) or the animals' responses within the first hour post-disbudding. Stilwell et al. (2008) showed that flunixin-meglumine with no regional anaesthesia did not control pain after paste disbudding, even if given in a pre-emptive way 1 h before procedure.

Although pain and distress caused by disbudding is probably temporary, and the long run benefits for welfare are, as said, manifest, there are ethical reasons for studying more humane approaches to caustic paste disbudding. In order to assess the level of pain experienced after caustic paste disbudding and to understand which anaesthesia/analgesia protocols are better for calves' welfare, three experiments were designed Experiment 1 evaluated pain from 1 to 24 h in calves disbudded with: both local anaesthesia and a non-steroidal-anti-inflammatory analgesic, anaesthesia only, or neither; Experiment 2 compared pain during the first hour post-disbudding in calves in the same three conditions as in Experiment 1; Experiment 3 to assess the duration of action of effective local anaesthesia with lidocaine 2%.

2. Material and methods

2.1. Farm and animals

All experiments were carried out in the same 500 milking-cow dairy farm. All female calves were kept in a group pen (~200 m^2) which consisted of a straw-bedded lying area and a solid floor feeding area. Animals were fed whole milk and concentrate from two computer-controlled feeding stations. Calves were accustomed to human proximity due to routine care.

2.2. Common procedures

Different numbers of calves were included in each disbudding session, depending on the availability of calves of similar size and with a small horn bud. At each disbudding session calves were randomly allocated to the different groups.

The caustic paste used for disbudding (sodium hydroxide, SD-plus®) was applied with a spatula after clipping the hair around the base of the horn. Sham-disbudded animals were handled and hair-clipped in the same way, but an obstetric gel (VetopGel®) was applied on the horn instead of the paste. All calves were coloured-marked on both flanks with a randomly chosen number for easier identification when behaviour was assessed.

Cornual nerve anaesthesia needed for disbudding, was achieved by the injection of 5 mL of 2% lidocaine, without adrenaline, just ventral to the lateral edge of the frontal bone, midway from the base of the horn to the lateral canthus of the eye (Noordsy, 1994). Nerve blocking was confirmed by needle pricking, 5 min after injection when the hair was clipped. When applicable, 5 mL of saline was injected s/c by the same technique.

Blood sampling (7 mL) into a heparinised tube was by left jugular venipuncture. Blood was kept in ice then centrifuged and plasma frozen (−20 °C). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). The lowest detectable concentration of cortisol was 1.0 nmol/L. The intra-assay coefficients of variation was 9.2% for 1 ng/mL and 3.3% for 5 ng/mL and the inter-assay coefficients of variation was 3.4%, 1.4% and 1.2%, for Experiments 1, 2 and 3, respectively (Rodbard, 1974). The between-day differences for plasma cortisol concentrations within groups were not significant so data were pooled.

The frequency of four behaviours was recorded, namely: head-shaking, ear-flicking, head-rubbing (with hind feet or against objects) and transitions (quick transition from standing to lying and back to standing). Additionally, the number of animals adopting the ''inert-lying'' postures was registered if the calf showed the already described behaviour for more than 30 s in each observational period.

All disbudding procedures were done at the same time of day, in similar weather conditions and by the same operator. Behaviour recording and blood sampling (jugular venipucture) were by an experienced veterinarian, blind to treatments.

2.3. Experiment 1

Thirty-two female Holstein calves (mean age 27 ± 8 days; estimated weight of 60–70 kg), were randomly allocated to four groups: PD_1—caustic paste (SH-Plus®) disbudded 5 min after saline injection (n = 8); PDA_1—paste-disbudded 5 min after 2% lidocaine injection (n = 9); $PDAF_1$—paste-disbudded 5 min after i.v. injection of 3 mL flunixin-meglumine (approximately 2.2 mg/kg) and 2% lidocaine (n = 7); SD_1—sham-disbudded 5 min after saline injection (n = 8). Of the original nine calves belonging to SD_1 group, one was removed from the study because of respiratory disease signs.

Blood was collected at 5 min before (baseline values) and 1, 3, 6 and 24 h after disbudding. The incidence of the five pain-related behaviours was recorded throughout 15 min periods just after disbudding and then before each blood sampling.

2.4. Experiment 2

Thirty-five female Holstein calves (mean age 22 ± 4 days) were randomly allocated to four groups: PD_2—caustic paste (SH-Plus®) disbudded 5 min after saline injection (n = 7); PDA_2—paste-disbudded 5 min after 2% lidocaine injection (n = 10); $PDAF_2$—paste-disbudded 5 min after i.v. injection of 3 mL of flunixin-meglumine (approximately 2.2 mg/kg) and 2% lidocaine (n = 10); SD_2—sham-disbudded 5 min after saline injection (n = 8). Blood was collected at 5 min before (baseline values) and 10, 30 and 50 min after disbudding. Behaviour was recorded for 10 min periods, just before each blood sampling.

2.5. Experiment 3

Sixteen female Holstein calves (mean age 28 ± 6 days) were randomly allocated to three groups: PD_3—caustic paste (SH-Plus®) disbudded 5 min after saline injection (n = 6); PDA_2—paste-disbudded 5 min after 2% lidocaine injection (n = 6); SD_2—sham-disbudded 5 min after saline injection (n = 4).

Blood was collected at 5 min before (baseline values) and 90, 120, 150 and 180 min after disbudding. Behaviour was recorded throughout 15 min periods just after disbudding and then before each blood sampling.

2.6. Statistical analysis

Distributions of the variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used. Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between groups at each time

Table 1
Mean ± S.D. plasma cortisol (nmol/L) of calves disbudded with caustic paste in Experiment 1

Group	Time from disbudding				
	−5 min	+1 h	+3 h	+6 h	+24 h
PDA_1 ($n = 9$)	12.07 ± 6.85^{aA}	32.88 ± 26.59^{aB}	18.37 ± 8.07^{aAB}	17.91 ± 12.61^{aAB}	16.62 ± 13.88^{aAB}
$PDAF_1$ ($n = 7$)	12.94 ± 10.27^{aA}	13.98 ± 11.49^{aA}	6.25 ± 5.74^{bA}	12.51 ± 9.63^{aA}	9.18 ± 8.56^{aA}
PD_1 ($n = 8$)	16.86 ± 11.15^{aA}	62.64 ± 10.32^{bB}	19.44 ± 14.14^{aA}	16.60 ± 18.41^{aA}	12.34 ± 12.05^{aA}
SD_1 ($n = 8$)	13.78 ± 9.81^{aA}	14.54 ± 9.25^{aA}	12.32 ± 12.32^{abA}	20.15 ± 13.88^{aA}	13.26 ± 14.09^{aA}

PDA_1: calves disbudded after treatment with lidocaine; $PDAF_1$: calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_1: calves disbudded without treatment; SD_1: calves sham-disbudded. Different lower case letters indicate difference between groups for which $p < 0.05$. Different uppercase letters indicate difference across time for which $p < 0.05$.

Table 2
Incidence (mean ± S.D.) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with caustic paste in Experiment 1

Group	Time from disbudding				
	+15 min	+1 h	+3 h	+6 h	+24 h
PDA_1 ($n = 9$)	2.67 ± 1.66^{a}	2.11 ± 1.54^{ab}	2.67 ± 2.00^{a}	3.78 ± 6.69^{a}	0.11 ± 0.03^{a}
$PDAF_1$ ($n = 7$)	3.57 ± 1.27^{a}	0.57 ± 0.79^{bc}	3.14 ± 3.53^{ab}	1.57 ± 2.94^{ab}	0.57 ± 0.53^{a}
PD_1 ($n = 8$)	6.38 ± 1.77^{b}	2.75 ± 1.83^{a}	1.88 ± 1.89^{ab}	0.50 ± 1.07^{ab}	0.13 ± 0.35^{a}
SD_1 ($n = 8$)	0.63 ± 0.74^{c}	0.13 ± 0.35^{c}	0.38 ± 0.52^{b}	0.13 ± 0.35^{b}	0.38 ± 0.52^{a}

Observational period: 15 min. Treatment groups: PDA_1—calves disbudded after treatment with lidocaine; $PDAF_1$—calves disbudded after treatment with lidocaine and analgesia (flunixin-meglumine); PD_1—calves disbudded without treatment; SD_1—calves sham-disbudded. Different lower case letters indicate difference between groups for which $p < 0.05$.

were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance.

3. Results

There was no difference in mean age between groups in any of the experiments. Sham-disbudded calves did not show any changes in plasma cortisol levels at any time during the three experiments.

3.1. Experiment 1

Cortisol results from Experiment 1 are shown in Table 1. There were no differences between groups in baseline values (−5 min) or at 6 h and 24 h after disbudding. At 1 h after the procedure, calves disbudded with no treatment (PD_1) showed higher cortisol compared with PDA_1 ($p = 0.006$) and with the other two groups ($p < 0.001$). At 1 h after disbudding PDA_1 calves showed numerically higher cortisol than $PDAF_1$, but this differences was not significant ($p = 0.055$). Only PD_1 ($p < 0.001$) and PDA_1 ($p = 0.015$) showed an increase at 1 h in relation to baseline. At 3 h $PDAF_1$ showed a lower cortisol level compared with PDA_1 and PD_1 (both $p = 0.005$) but equal to sham-disbudded animals.

The incidence of all pain-related behaviours (mean ± S.D.) recorded during Experiment 1, is presented in Table 2. Compared with sham-disbudded animals: PD_1 showed more behaviours at 15 m and 1 h (both $p > 0.001$); PDA_1 showed a higher incidence of pain-related behaviours at 15 min ($p = 0.008$), 1 h ($p = 0.008$), 3 h ($p = 0.006$) and 6 h ($p = 0.015$); $PDAF_1$ only showed more pain-related behaviours at 15 min ($p < 0.001$). The non-treated disbudded calves showed more behaviours than PDA_1 ($p = 0.001$) and $PDAF_1$ ($p = 0.004$) at 15 min, but at 1 h only differed from $PDAF_1$ ($p = 0.009$). The behaviours more commonly recorded after paste disbudding were head-shaking and head-rubbing at 15 min and 1 h (data not shown). At 3 h the "inert-lying" behaviour was recorded in three animals from the PDA_1 and four animals from the PD_1 group (Fig. 1).

3.2. Experiment 2

No differences were found between groups in cortisol baseline values or at 10 min after disbudding (Table 3). At

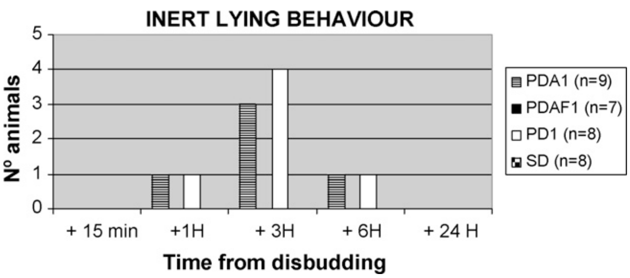

Fig. 1. Number of animals showing "inert-lying" behaviour after caustic paste disbudding in Experiment 1. PDA_1: calves disbudded after treatment with lidocaine; $PDAF_1$: calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_1: calves disbudded without treatment; SD_1: calves sham-disbudded.

Table 3
Plasma cortisol (mean ± S.D.) of calves disbudded with caustic paste in Experiment 2

Group	Time from disbudding			
	−5 min	+10 min	+30 min	+50 min
PDA_2 (n = 10)	11.16 ± 7.9^{aA}	19.11 ± 11.40^{aA}	16.71 ± 10.69^{aA}	14.73 ± 8.80^{aA}
$PDAF_2$ (n = 10)	18.92 ± 13.71^{aA}	23.14 ± 16.67^{aA}	20.67 ± 12.98^{aA}	19.80 ± 9.67^{aA}
PD_2 (n = 7)	17.49 ± 12.92^{aA}	25.54 ± 15.15^{aAB}	41.39 ± 14.85^{bBC}	42.32 ± 14.47^{bC}
SD_2 (n = 8)	15.26 ± 6.13^{aA}	16.84 ± 7.06^{aA}	20.20 ± 11.19^{aA}	14.34 ± 8.57^{aA}

PDA_2: calves disbudded after treatment with lidocaine; $PDAF_2$: calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_2: calves disbudded without treatment; SD_2: calves sham-disbudded. Different lower case letters indicate difference between groups for which $p < 0.05$. Different uppercase letters indicate difference across time for which $p < 0.05$.

Table 4
Incidence (mean ± S.D.) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with caustic paste in Experiment 2

Group	Time from disbudding		
	0–10 min	20–30 min	40–50 min
PDA_2 (n = 10)	1.60 ± 0.97^{a}	0.60 ± 0.52^{a}	1.60 ± 1.26^{a}
$PDAF_2$ (n = 10)	1.30 ± 0.82^{ac}	0.50 ± 0.53^{ac}	0.50 ± 0.53^{b}
PD_2 (n = 7)	4.14 ± 0.69^{b}	3.43 ± 1.62^{b}	3.86 ± 1.46^{c}
SD_2 (n = 8)	0.63 ± 0.52^{c}	0.00 ± 0.00^{c}	0.25 ± 0.46^{b}

Observational periods: 10 min. Treatment groups: PDA_2—calves disbudded after treatment with lidocaine; $PDAF_2$—calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_2—calves disbudded without treatment; SD_2—calves sham-disbudded. Different lower case letters indicate difference between groups for which $p < 0.05$.

30 and 50 min after disbudding the PD_2 group showed a higher cortisol level compared with baseline (p = 0.028) and to all other groups (all $p < 0.05$).

The incidence of pain-related behaviours (mean ± S.D.) observed during Experiment 2, is presented in Table 4. PD_2 showed more pain signs ($p < 0.001$, except PDA_2 p = 0.005) at all observation times compared with the other three groups. The PDA_2 showed more behaviours than sham-disbudded animals during the first (p = 0.034), second (p = 0.034) and third (p = 0.016) period of observation and more than $PDAF_2$ (p = 0.035) at 50 min. $PDAF_2$ showed the same behaviours as sham-disbudded animals at all times. The behaviours seen in disbudded animals were mainly head-shaking and head-rubbing. Sham-disbudded calves only showed ear-flicking. No inert lying was recorded during this experiment.

3.3. Experiment 3

Plasma cortisol variations from Experiment 3 are presented in Table 5. Baseline cortisol levels were equal between groups. Calves disbudded with no treatment showed higher than baseline levels at 90 min (p = 0.046) and 180 min (p = 0.028). The PDA_3 showed an increase in cortisol at 180 min (p = 0.028) compared with baseline. At 90 min PD_3 showed higher levels than SD_3 (p = 0.038) but at 180 min it was the PDA_3 group that had higher cortisol

Table 5
Plasma cortisol (mean ± S.D.) of calves disbudded with caustic paste in Experiment 3

Group	Time from disbudding				
	−5 min	+90 min	+120 min	+150 min	+180 min
PDA_3 (n = 6)	13.7 ± 12.4^{aAB}	23.3 ± 18.6^{aABC}	5.8 ± 7.6^{aA}	28.1 ± 15.7^{aBC}	43.3 ± 9.8^{aC}
PD_3 (n = 6)	14.9 ± 8.4^{aA}	40.5 ± 17.7^{bB}	11.9 ± 16.4^{aAB}	20.1 ± 8.5^{aAB}	27.2 ± 5.3^{bB}
SD_3 (n = 4)	12.7 ± 12.3^{aA}	15.7 ± 9.9^{aA}	12.8 ± 12.9^{aA}	22.4 ± 6.2^{aA}	16.5 ± 14.3^{bA}

PDA_3: calves disbudded after treatment with lidocaine; PD_3: calves disbudded without treatment; SD_3: calves sham-disbudded. Different lower case letters indicate difference between groups for which $p < 0.05$. Different uppercase letters indicate difference across time for which $p < 0.05$.

Table 6
Incidence (mean ± S.D.) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with caustic paste in Experiment 3

Group	Time from disbudding			
	90 min	120 min	150 min	180 min
PDA_3 (n = 6)	1.17 ± 0.98^{a}	2.17 ± 1.47^{ab}	2.17 ± 1.94^{ab}	3.83 ± 2.86^{a}
PD_3 (n = 6)	4.33 ± 2.58^{b}	2.33 ± 1.37^{a}	2.83 ± 1.05^{a}	0.83 ± 0.75^{ab}
SD_3 (n = 4)	1.00 ± 0.82^{a}	0.25 ± 0.50^{b}	0.75 ± 0.96^{b}	0^{b}

Observational periods: 15 min. Treatment groups: PDA_3—calves disbudded after treatment with lidocaine; PD_3—calves disbudded without treatment; SD_3—calves sham-disbudded. Different lower case letters indicate difference between groups for which $p < 0.05$.

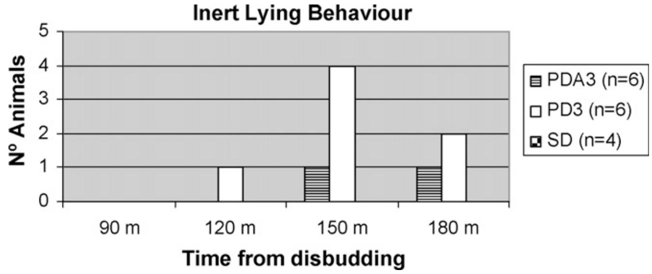

Fig. 2. Number of animals showing "inert-lying" behaviour after caustic paste disbudding in Experiment 3. PDA_3: calves disbudded after treatment with lidocaine; PD_3: calves disbudded without treatment; SD_3: calves sham-disbudded.

levels than PD_3 ($p = 0.004$) and SD_3 ($p = 0.038$). At 120 and 150 min there were no cortisol differences between groups.

The incidence of pain-related behaviours observed during Experiment 3, are presented in Table 6. Non-treated disbudded calves showed more behaviours than sham-disbudded at 90 ($p = 0.019$), 120, 150 (both $p = 0.038$) but not at 180 min ($p = 0.114$). Animals treated with local anaesthesia (PDA_3) showed less behaviours at 90 min ($p = 0.015$) when compared with group PD_3 and more at 180 min ($p = 0.041$) when compared with sham-disbudded calves ($p = 0.038$). At 180 min after disbudding PDA_3 calves showed numerically more behaviours than PD_3, but this difference was not significant ($p = 0.065$). The number of animals in each group showing "inert lying" is presented in Fig. 2.

4. Discussion

The division of the study into three separate experiments allowed the need for the second and third studies to be determined by analysing the results of the previous experiment. Also calf stress, due to frequent handling and blood sampling, was reduced by using three sets of animals.

No statistically significant changes were found in plasma cortisol or in behaviour where calves were sham-disbudded. This suggests that handling did not affect these measures.

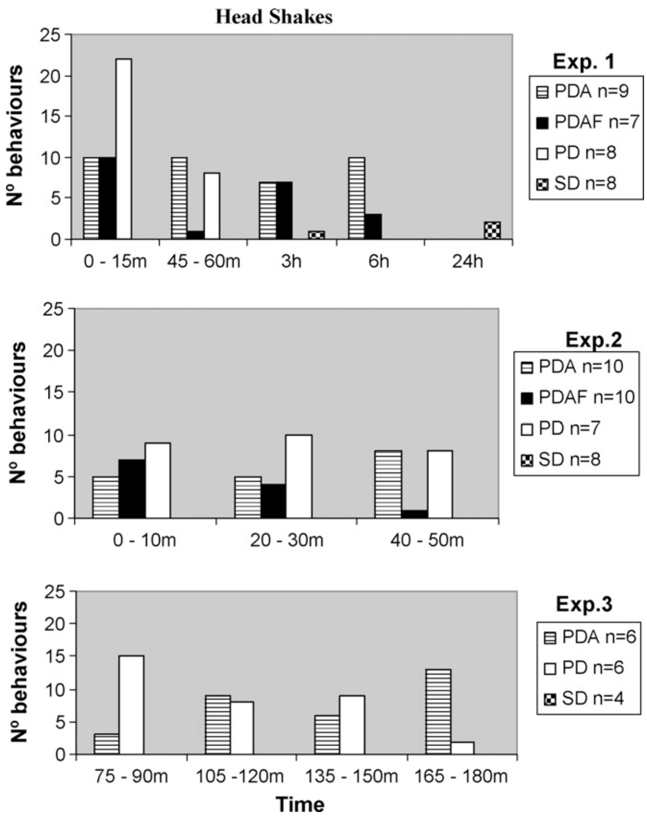

Fig. 3. Incidence of "head shakes" for 1-month-old calves disbudded with caustic paste in three different experiments. PD: paste-disbudded; PDA: paste-disbudded with local anaesthesia; PDAF: paste-disbudded with local anaesthesia and analgesia (flunixin-meglumine); SD: sham-disbudded.

Cortisol levels in Experiment 1 suggest that paste disbudding with no treatment only causes pain at 1 h. However, the incidence of pain-related behaviours at 3 h, in animals disbudded without analgesia, shows that some discomfort is felt until a later period. Experiment 3 also shows a high incidence of pain behaviours until 3 h and a very high level of cortisol at 90 min, compared with baseline and sham-disbudded calves. Morisse et al. (1995) and Vickers et al. (2005) also showed a high number of pain-related behaviours up to 4 h, although Vickers' study was done with animals sedated with xylazine. All these results suggest that caustic paste disbudding causes distress in young calves for, at least, the first 3 h.

Graf and Senn (1999) showed a marked and immediate rise in plasma cortisol that peaked (80 nmol/L) 20 min after hot-iron disbudding. Likewise, Stafford et al. (2003) found that cortisol increased rapidly and peaked at 30 min (90 nmol/L) after scoop-dehorning. Petrie et al. (1995) and Grøndahl-Nielsen et al. (1999) also showed plasma cortisol rises immediately after the procedures with hot-iron, suggesting intense and immediate pain. This is to be expected due to the strong restraint needed and the extensive and sudden damage of the tissues. The time from the application of caustic paste to the onset of measurable pain was only assessed in one study (Vickers et al., 2005) but was done in xylazine-sedated animals. In Experiment 1 we show that the highest incidence of pain-behaviours is seen almost immediately (0–15 min) after the disbudding suggesting that pain is felt very soon after the procedure. The results from Experiment 2 are in agreement with this suggestion because it shows behaviour modification soon after the procedure and a cortisol rise at 10 min, although it only becomes significant, compared with baseline and sham-disbudded animals, at 30 and 50 min. This suggests that behaviour analysis is a better indicator of very recent pain-induced distress possibly because the cortisol response is delayed.

The first two experiments show that plasma cortisol rises continuously reaching its highest level at 60 min after dehorning, in contrast to animals disbudded with hot-iron that show the highest cortisol level at 30 min (Doherty et al., 2007). This suggests that paste disbudding causes pain very soon after being in contact with the tissues, but is slower in triggering full nociceptor activity, probably due to the fact that caustic burns with strong bases, in contrast with the temporary activity of thermal burns, continues to cause damage as long as the active chemical is in contact with the tissue (Yano et al., 1993).

In contrast to other studies (Vickers et al., 2005), the present study shows that 5 mL of 2% lidocaine injected on the cornual nerve is efficient in reducing, but not preventing, the cortisol rise and pain-related behaviours that are seen in non-treated animals, for 1 h. However, the anaesthesia used in Vickers' study (1.5 mL to block the

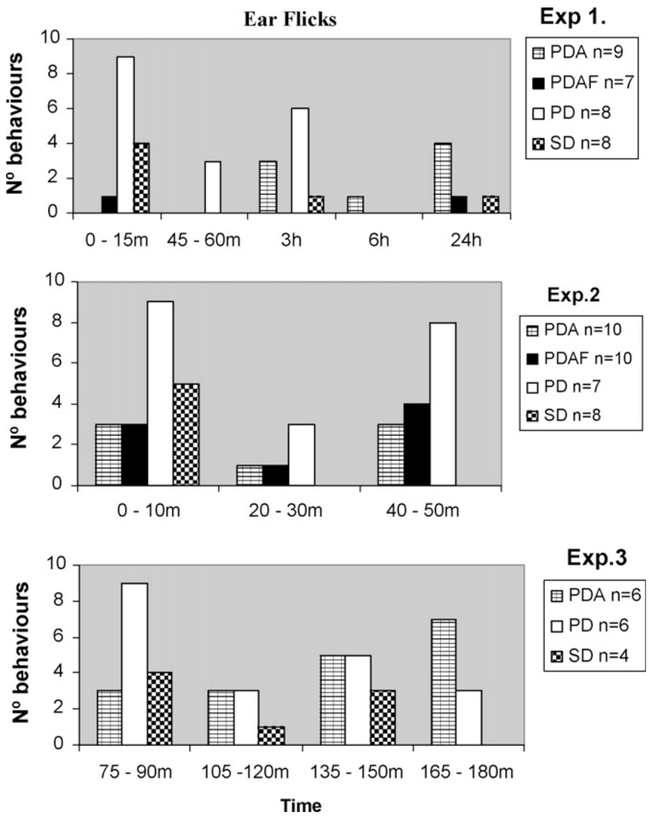

Fig. 4. Incidence of "ear flicks" for 1-month-old calves disbudded with caustic paste in three different experiments. PDA: paste-disbudded with local anaesthesia; PDAF: paste-disbudded with local anaesthesia and analgesia (flunixin-meglumine); PD: paste-disbudded; SD: sham-disbudded. In Experiment 1 recording at 3, 6 and 24 h were equally done for a period of 15 min.

cornual nerve and 3 mL s/c at the base of the horn) may not have been the more adequate, as was admitted by the authors. The control of pain in our study was incomplete during the first hour, for there were more behaviours than sham-disbudded and higher cortisol than baseline at 1 h, and was temporary since there was higher cortisol at 180 min and higher incidence of pain-related behaviours from 1 to 6 h as compared with sham-disbudded animals. This suggests that nerve-blocking does offer some protection to the calf disbudded with caustic paste but is not totally efficient and when it subsides additional discomfort is felt. Similar results have been shown after hot-iron and scoop-disbudding (Petrie et al., 1995; McMeekan et al., 1998; Sutherland et al., 2002; Stafford et al., 2003; Stilwell and Lima, 2004), with cortisol and pain-related behaviours rising 1 or 2 h after the use of lidocaine regional anaesthesia.

The first two experiments of the present study show that flunixin-meglumine associated with the lidocaine block prevents the cortisol rise and pain-related behaviours. This suggests that the association of the two drugs is efficient in blocking early and intense stimuli arising from the chemically burned area. However, more behaviours related to pain (head shaking and head rubbing), are seen in the group disbudded after treatment with flunixin-meglumine (Figs. 3 and 5) compared with sham-disbudded animals that just show ear-flicking (Fig. 4) probably because of handling, hair-clipping or the contact of the gel with the skin. This difference indicates that some noxious sensations arise in disbudded and analgesic-treated animals during the first minutes of paste activity.

The three experiments show that the incidence of pain-related behaviours (Figs. 3–6) is a useful indicator of pain in calves disbudded with caustic paste and should be used together with cortisol assessment. Our results show that head-shakes and head-rubs (Figs. 3 and 5), especially rubbing the head with the hind feet, are the most common behaviours in calves disbudded with caustic paste and no pain-relief treatment. Ear flicks (Fig. 4) were not easy to detect in those animals that were constantly shaking or rubbing their heads and this may explain the relatively low incidence of a very easy to perform behaviour. In contrast, this is the only behaviour that sham-disbudded animals show just after the procedure. Grøndahl-Nielsen et al. (1999) found more ear-flicks in hot-iron disbudded calves compared with sham-disbudded but this could have been a result of a different type of tissue damage. In the present study, had this behaviour (ear-flicks) not been included, differences between disbudded and not-disbudded groups would have been greater. Mellor et al. (2005) suggest that behaviours that are shown by treated animals but not observed in controls or in animals subjected to some form of analgesia, is likely to be a useful index of noxious

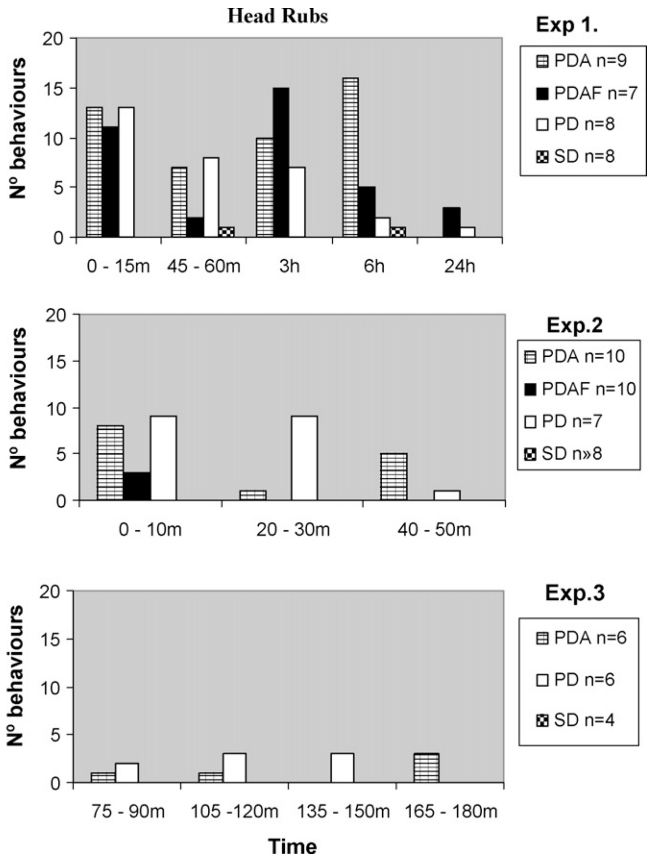

Fig. 5. Incidence of "head rubs" for 1-month-old calves disbudded with caustic paste in three different experiments. PDA: paste-disbudded with local anaesthesia; PDAF: paste-disbudded with local anaesthesia and analgesia (flunixin-meglumine); PD: paste-disbudded; SD: sham-disbudded.

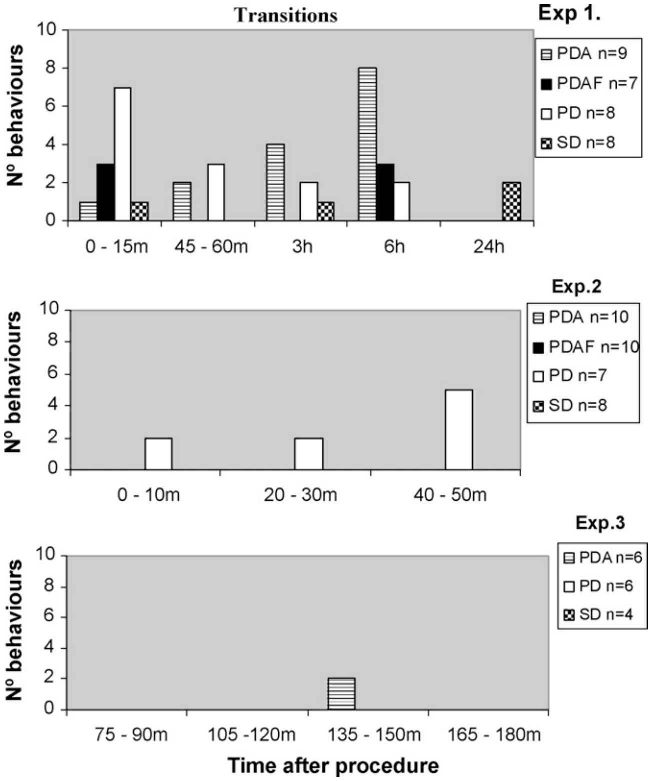

Fig. 6. Incidence of "transitions between lying and standing" behaviour for 1-month-old calves disbudded with caustic paste in three different experiments. PDA: paste-disbudded with local anaesthesia; PDAF: paste-disbudded with local anaesthesia and analgesia (flunixin-meglumine); PD: paste-disbudded; SD: sham-disbudded.

sensory input leading to pain and distress. Accordingly we suggest that ear-flick should not be used in studies on pain-induced distress after paste disbudding because of the risk of hiding important differences.

The results of the present study shows that "inert lying" is an important behaviour that should be used to assess pain in young calves disbudded with caustic paste. "Inert lying" was never recorded during Experiment 2 but seen in several animals from Experiment 1 and 3 (Figs. 1 and 2, respectively). These results indicate that this behaviour relates to the intense distress felt during the first few hours after the caustic burn. However, this behaviour was not shown by animals in studies on scoop (Stilwell, 2008) and hot-iron dehorning (Stilwell, 2004) even in animals showing very high cortisol values. The difference in age (younger in paste-disbudded animals) or the type of tissue damage, as suggested by Mellor et al. (2005), may be responsible for the absence of this motionless state of calves. While assuming the inert-lying behaviour, the calves did not show any of the other monitored behaviours. By keeping almost immobile for the entire observational periods, four animals in the PD_1 group caused a reduction in the other recorded behaviours at 3 h, so that no difference was found when compared with sham-disbudded calves (Table 2). A similar effect occurred for the PDA_3 and PD_3 at 150 min (Table 6). Neglecting the significance of inert lying may lead to erroneous conclusions when evaluating the intensity and duration of distress in young calves.

5. Conclusions

Caustic paste disbudding causes intense pain from the first minutes after paste application and some behavioural signs of distress still remain at 3 h after the procedure. Behavioural analyses indicated that pain and distress are felt in calves treated with local anaesthesia and flunixin-meglumine during the first minutes after paste disbudding, although no significant increase in cortisol was shown. Treatment with local anaesthesia alone does control pain for the first hour but not from then to 6 h post-disbudding. We also suggest that inert-lying behaviour is a useful indicator in studies of pain in young calves although the further studies are needed to assess the relation between this behaviour and the intensity of pain.

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